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(54) Title: MODIFIED PECTIC SUBSTANCE

(57) Abstract: Modified pectic substance having emulsifying, microencapsulating, foam stabilizing and/or film forming properties, obtainable by treating a pectic substance with a proteolytic enzyme selected from the group consisting of proteases and peptidases, and products consisting of or containing such modified pectic substance.

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Modified pectic substance

FIELD OF INVENTION

The present invention relates to a modified pectic substance, a process of modifying a pectic substance, products containing a modified pectic substance, an emulsifier, a foam stabilizer, a film forming agent, a microencapsulating agent, and a process of preparing an emulsified product.

BACKGROUND OF THE INVENTION

Emulsions, i.e. disperse systems consisting of two or more mutually insoluble or sparingly soluble liquids and in which one liquid - the continuous or external phase - usually is present in excess relative to the second liquid - the dispersed or internal phase - find wide spread use in the food, beverage and pharmaceutical industries.

The manufacture of such products normally involve two problems, i.e. droplet formation to form an emulsion and stabilization of the emulsion formed, and it is well-known to use agents, viz. emulsifiers, emulsion promoters and emulsion stabilizers to boost the emulsifying process.

The emulsifiers presently used may be divided into three groups, viz. (1) synthetic surface-active emulsifiers, (2) emulsifiers of natural origin, and (3) inorganic emulsifiers that generally have a low surface activity.

Most synthetic surface-active emulsifiers are unacceptable for use in products for human intake such as food products and beverages, and, therefore, natural emulsifiers are preferable for use in such products.

Some of the natural emulsifiers such as whey proteins, e.g. casein, are of animal origin and are unacceptable for use in vegetarian foods, kosher foods and halal foods, and others such as gum arabic can only be obtained in limited amounts.

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Therefore, it has been attempted to find other natural non-animal emulsifiers such as pectin for use in emulsified food products, beverages and similar products for human intake.

US patent No. 5 900 268 (Mazoyer et al.) discloses depolymerised citrus fruit, sugar beet pectin and apple pectins and their use as emulsifiers and emulsion stabilizers.

EP 426.434 A1 discloses the use of unmodified sugar beet pectin in food or drug comestibles, e.g. whipped products, emulsions, or gels.

WO 97/10726 A1 discloses a process for increasing the viscosity or gel strength of food products by subjecting a pectinaceuos homogenate or slurry from fruit or vegetables, e.g. orange, broccoli, or tomato, to an enzymatically treatment by a mixture of enzymes.

EP 580.252 A2 discloses a novel class of pectin methyl esterases present in certain plant enzyme extracts, in particular papain, ficin and bromelain. The enzymes reduce the degree of methoxylation of certain pectins, such as apple pectin.

The article "Acetylated pectic polysaccharides of sugar beet", Dea et al., Food Hydrocolloids, Vol. 1, No. 1, pp. 71-88, 1986, presents a study of the chemical composition and the surface-active and emulsifying properties of sugar beet pectin. One of the purposes was to find out whether contaminating protein associated with the pectin is contributing to foaming properties of unmodified pectin. By treating sugar beet pectin with papain, all the protein associated with the sugar beet was removed. It can be concluded form the article that foaming properties were due to the pectin polysaccharide itself and not to the contaminating protein.

The object of the present invention is to modify a pectic substance from beetroot to improve its emulsifying and foaming properties and make it

suitable for use in emulsified products for human intake as well as cosmetics and health care products, such as lotions and cremes.

SUMMARY OF THE INVENTION

The modified pectic substance according to the invention is characterized in that it is obtainable by treating a pectic substance from beetroot with a proteolytic enzyme selected from the group consisting of proteases and peptidases.

It is well known that pectic substances may contain minor amounts of proteins, e.g. in an amount of about 1-5% w/w. However, it is surprising that the emulsifying properties of e.g. pectin are significantly improved by treating pectin with a proteolytic enzyme selected from a group consisting of proteases and peptidases.

As used in connection with the present invention, the term "pectic substance" encompasses pectin, pectic acid and salts and esters of pectic acid (pectates), whereby the pectic substance has a galacturonic acid content of above 40 %.

The galacturonic acid content of the pectic substance is preferable above 50 % and more preferred above 65 %.

The pectic material to be modified according to the invention is derived from beetroot (Beta vulgaris L. Chenopodiaceae), including sugar beet, garden beets (red beets), chard, mangel, spinach beet, silver beet, and fodder beet. Sugar beet pectin is a particularly useful pectic substance.

Proteases and peptidases, which are classified in, class E.C.3.4. of the Enzyme Classification System described in Enzyme Nomenclature 1992, Academic Press, San Diego, California, with supplements, catalyse the hydrolyses of peptide bonds in proteins.

Preferred proteolytic enzymes are endopeptidases, such as papain and pepsin.

Other preferred proteolytic enzymes are exopeptidases, such as carboxypeptidase B.

Preferred enzymes are also mixtures of endo- and exopeptidases.

Examples of suitable commercial proteases are Papain 16000 from Valley Research, Collupulin® from DSM Gist-Brocades Food Specialities, and Flavorzyme™ 500 L from Novozymes.

The present invention also relates to a process of modifying a pectic substance, said method comprising the steps of preparing a liquid medium containing a pectic substance from beetroot and adding to said medium a proteolytic enzyme selected from a group consisting of proteases and peptidases.

In practice, the modification of the pectic substance may be carried out as follows:

Temperature and pH of the pectin preparation is adjusted to working temperature and pH of the enzyme to be used. Enzyme is dissolved/diluted in ion exchanged water and added to the pectin preparation. Reaction is carried out while stirring continuously, and if necessary pH is controlled by titration. After a certain time reaction is terminated by lowering pH. In order to irreversibly inactivate the enzyme, temperature is raised to 80°C for 10 min. Temperature of the solution is lowered and the pectin is precipitated in 80% 2-propanol (using about 1 part of solution to about 3 parts of 80% 2-propanol). The precipitated pectin is drained on a belt press and put in a drying cabinet at 70°C for 24 hours. After drying the pectin is ground and sieved (DIN 24).

The pectin preparation to be used as substrate for enzyme treatment can be an extract directly obtained from the raw material e.g. sugar beet peel or it can be a solution of a refined pectin product.

An extract of sugar beet pectin may be prepared as follows:

1) Mixing dry granular beet pulp with an aqueous solution of a strong, mineral acid, preferably nitric acid
2) Extracting the pulp with rigorous agitation for about one to five hours at 60-80 °C and pH ranging from 1.5 to 2.5.
3) Separating the resulting mixture into waste solids and a liquid containing pectin
4) Treating the liquid containing pectin with enzyme as described above.

Pectin solution is made by adding pectin powder to hot (70°C) ion-exchanged water. The preparation is stirred continuously, until the pectin is completely dissolved.

The invention also relates to an emulsifier obtainable by modifying a pectic substance as described above.

The terms "emulsifier" and "emulsifying agent" as used herein are intended to mean products which exhibit emulsion forming or emulsion stabilizing properties or both.

In addition to its excellent emulsifying properties the modified pectic substances according to the invention exhibit foam stabilizing and film forming properties.

The present invention also relates to a process of preparing an emulsified product, said process being characterized in the use of a modified pectic

substance as emulsifier, wherein the modified pectic substance is obtainable by treating a pectic substance from beetroot with a proteolytic enzyme selected from the group consisting of proteases and peptidases.

The modified pectic substances according to the invention are suitable for use in a vast number of products such as food emulsions, e.g. soft drinks, margarine, ice cream, organic coffee milk, mayonnaise, salad dressings, bread, confectionary, pharmaceutical products, and cosmetics, and health care products, such as lotions and crèmes.

The modified pectic substance is also suitable for use as a microencapsulating agent. The term "microencapsulating" as used herein means a process of providing particles, each comprising a matrix of an encapsulating agent having embedded therein a plurality of solid or liquid micro particles.

For further information concerning the use of the modified pectic substance as a microencapsulating agent reference is made to the co-pending WO application No. ..... (Our reference No. P200100040 WO).

The invention will be described in further detail with reference to the following examples.

EXAMPLES

In the following examples the molecular weight (MW) is measured by the Capillary Tube Method principle as follows:

The outlet time is measured for a pectin/hexametaphosphate solution and the molecular weight is thereafter calculated after a well-known formula (see WO 00/58367 Pectin having reduced calcium sensitivity, page 12).

The outlet time is measured on two outlets. If the difference between the times is more than 0.4 seconds the measuring is repeated until the difference

is less than the appropriate value. The outlet time used for the molecular determination is the mean value of the above-mentioned identical or substantially identical measuring results.

Preparation of enzymatically modified pectic substances

Example 1

In this example the pectic substance was derived from sugar beets (GENU® beta pectin, lot 92455, produced by CP Kelco, Lille Skensved, Denmark) and the enzyme was papain (Collupulin® batch R9741, produced by DSM Gist-Brocades Food Specialities, Delft, The Netherlands).

1000 l of ion-exchanged water was heated to 70 °C, 0.4 M of NaCl was dissolved and 20 kg of pectic substance was added, while stirring continuously. After the pectin was completely dissolved, the temperature was lowered to 45 °C; pH was adjusted to 5.50 by titration with a 2% (w/v) NH_3 solution. 240 grams of Collupulin was dissolved in approximately 5 l ion exchanged water at ambient temperature and added to the pectin solution. During the experiment pH was kept constant at 5.50 by titration with 2% (w/v) NH_3. After 20 minutes, 6127 ml of 2% (w/v) NH_3 was added, and the reaction was stopped by addition of a 10% (w/v) HNO_3 solution till pH 2.50. In order to irreversibly inactivate the enzyme, the temperature was raised to 80 °C. After 10 minutes at 80 °C, the solution was cooled to 50 °C and the modified pectin was precipitated (1:3) in 80% 2-propanol. The precipitated pectin was drained on a belt press and put into a drying cabinet at 70 °C for 24 hours. After drying the pectin was ground and sieved (DIN 24). Degree of acetylation (%D(Ac)), degree of esterification (%DE), galacturonic acid content (%GA) and molecular weight (MW) of the enzyme treated pectin were determined and the results obtained appear from Table 1.

TABLE 1

	Collupulin treated sugar beet pectin
%D(Ac)	19.9
%DE	38.8
%GA	76.9
MW	63000 Da

Example 2

In this example the pectic substance was derived from sugar beet (GENU® beta pectin, lot 92455, produced by CP Kelco ApS, Lille Skensved Denmark) and the enzyme was pepsin (P 6887 from Sigma Aldrich. Lot 99H7665)

50 l of ion-exchanged water was heated to 70 °C, and 2 kg of pectic substance was added stirring continuously. After the pectin was completely dissolved, temperature was lowered to 40 °C and pH was adjusted to 2.5 by titration with a 2 % (w/v) HNO_3 solution.

2,1 grams of the pepsin were dissolved in 100 ml ion exchanged water and added to the pectin solution. During the experiment the pH was kept constant by titration with 2% (w/v) NH_3. After 4 hours the reaction was stopped by addition of a 10 % (w/v) HNO_3 solution till pH 3.8-4.0. In order to irreversibly inactivate the enzyme, temperature was raised to 80 °C. After 10 minutes at 80 °C, the solution was cooled to 50 ° C and adjusted to pH 2.5 with 2% (w/v) HNO_3. The pectin in the solution was then precipitated (1:3) in 80% 2-propanol. The precipitated pectin was drained on a belt press and put in a

drying cabinet at 70 °C for 24 hours. After drying the pectin was ground and sieved (DIN 24). Degree of acetylation (%D(Ac)), degree of esterification (%DE), galacturonic acid content (%GA) and molecular weight (MW) of the enzyme treated pectin was determined, and the results obtained appear from Table 2.

TABLE 2

	Pepsin treated sugar beet pectin
% D(Ac)	19.0
%DE	53.4
%GA	72.0
MW	61110 Da

Example 3

In this example the pectic substance was derived form sugar beets (GENU® Beta pectin, lot 30003, produced by CP Kelco, Grossenbrodde, Germany), and the enzyme was Carboxypeptidase B from Sigma, Batch no. 108H7406, activity 176u/mg.

40 l of ion-exchanged water was heated to 70 °C and 1,6 kg of pectic substance was added, while stirring continuously. After the pectin was completely dissolved, the temperature was lowered to 45 °C; pH was adjusted to 7.50 by titration with a 10% (w/v) soda solution. 21 mg of Carboxypeptidase B was added to the pectin solution. During the experiment the pH was kept constant at 7,5 by titration with 5% (w/v) soda. After 24 hours the reaction was stopped by addition of a 10% (w/v) soda solution till pH 2,50. In order to irreversibly inactivate the enzyme, the temperature was raised to 80 °C. After 10 minutes at 80 °C, the solution was cooled to 50 °C and the modified pectin was precipitated (1:3) in 80% 2-propanol. The

precipitated pectin was drained on a belt press and put into a drying cabinet at 70 °C for 24 hours. After drying, the pectin was ground and sieved (DIN 24). Degree of acetylation (% D(Ac)), degree of esterification (% DE), galacturonic acid content (%GA) and molecular weight (MW) of the enzyme treated pectin were determined, and the results obtained appear from Table 3.

TABLE 3

	Carboxypeptidase B treated sugar beet pectin
% DAc	24,6
% DE	51,8
% GA	72,9
MW	69.100 Da

Example 4

In this example the pectic substance was derived from sugar beets (GENU® Beta pectin, lot 30003, produced by CP Kelco, Grossenbrodde, Germany), and the enzyme was Flavorzyme™ 500L from Novozymes, Denmark, Batch no. HPN01200 with an activity of 500 LAPU/g.

50 l of ion-exchanged water was heated to 70 °C, and 2 kg of pectic substance was added, while stirring continuously. After the pectin was completely dissolved, the temperature was lowered to 45 °C; pH was adjusted to 5.50 by titration with a 10% (w/v) soda solution. 15 ml of Flavorzyme 500L was added to the pectin solution. During the experiment the pH was kept constant at 5,5 by titration with 5% (w/v) soda. After 4 hours the reaction was stopped by addition of a 10% (w/v) soda solution till pH 2,50. In order to irreversibly inactivate the enzyme, the temperature was

raised to 80 °C. After 10 minutes at 80 °C, the solution was cooled to 50 °C and the modified pectin was precipitated (1:3) in 80% 2-propanol. The precipitated pectin was drained on a belt press and put into a drying cabinet at 70 °C for 24 hours. After drying, the pectin was ground and sieved (DIN 24). Degree of acetylation (% D(Ac)), degree of esterification (% DE), Galacturonic acid (%GA) and molecular weight (MW) of the enzyme treated pectin were determined, and the results obtained appear from Table 4.

TABLE 4

	Flavorzyme treated sugar beet pectin
% DAc	22.8
% DE	55.0
% GA	73.8
MW	61.000 Da

Example 5

In this example the pectic substance was derived from sugar beets (GENU® Beta pectin, lot 30003, produced by CP Kelco, Grossenbrodde, Germany) and the enzyme was Carboxypeptidase A from Sigma, Batch no. 127H7445, activity 50u/mg.

40 l of ion-exchanged water was heated to 70 °C and 1,6 kg of pectic substance was added, while stirring continuously. After the pectin was completely dissolved, the temperature was lowered to 45 °C; pH was adjusted to 7.50 by titration with a 10% (w/v) soda solution. 21 mg of Carboxypeptidase A was added to the pectin solution. During the experiment the pH was kept constant at 7,5 by titration with 5% (w/v) soda. After 24 hours the reaction was stopped by addition of a 10% (w/v) soda solution till

pH 2,50. In order to irreversibly inactivate the enzyme, the temperature was raised to 80 °C. After 10 minutes at 80 °C, the solution was cooled to 50 °C and the modified pectin was precipitated (1:3) in 80% 2-propanol. The precipitated pectin was drained on a belt press and put into a drying cabinet at 70 °C for 24 hours. After drying, the pectin was ground and sieved (DIN 24). Degree of acetylation (% D(Ac)), degree of esterification (% DE), content of galacturonic acid (%GA) and molecular weight (MW) of the enzyme treated pectin were determined, and the results obtained appear from Table 5.

TABLE 5

	Carboxypeptidase A treated sugar beet pectin
% DAc	17,8
% DE	37,5
% GA	74,6
MW	14.100 Da

Emulsion Test: Vitamin emulsions

In order to compare the emulsifying properties of various enzymatic treated beta pectins an emulsion test was conducted and particle size (oil droplet size) measured. The emulsion contained 20% dry matter and 80 % demineralised water.

Comparative Example 1

A 600 ml beaker with 365 ml boiling demineralised water was placed in a water bath at 80°C. 22.8 grams of sugar beet pectin (GENU® beta pectin

type BETA, lot 92455, from CP Kelco, Lille Skensved Denmark) was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 37.3 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 grams hot Vitamin E acetate oil (DL-α-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the freshly made emulsion was measured at 75 °C and found to be 252 cP. Particle size distribution (Malvern Mastersizer E, focal length 45 mm, presentation 0606, model independent, beam length 2.2 mm, specifications ±5μm) was measured. Mean oil droplet size, d(0.5), was found to be 1.70 μm and d(0.9) to be 3.78 μm.

To verify the emulsion stability the oil droplet size was also measured after 24 hours at 70 °C. The mean droplet size d(0.9) was found to be 3.74 μm.

Example 6

A 600 ml beaker with 365 ml boiling demineralised water was placed in a water bath at 80 °C. 22.8 grams of Collupulin modified sugar beet pectin as described in Example 1 was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 37.3 grams of saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 grams of hot Vitamin E acetate oil (DL-α-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the freshly made emulsion was measured at 75 °C and found to be 255 cP. Particle size distribution (Malvern Mastersizer E, focal length 45 mm, presentation 0606, model independent, beam length 2.2 mm, specifications ±5μm) was measured. Mean oil droplet size, d(0.5), was found to be 0.71 μm and d(0.9) to be 2.04 μm.

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To verify the emulsion stability the oil droplet size was also measured after 24 hours at 70 °C. The mean droplet size d(0.9) was found to be 2.35 µm.

As will appear from these results the oil droplet size was significantly smaller and hence the emulsifying properties (including the emulsion stabilizing properties) of the composition of the invention were far superior to those of the composition of Comparative Example 1.

Comparative Example 2

This example was conducted as Comparative Example 1 until the step of measurement of oil droplet size and distribution, where different equipment was used:

The freshly made emulsion of GENU® beta pectin type BETA, lot 92455 from CP Kelco, Lille Skensved, Denmark was measured at 75 °C and found to be 318 cP. Oil droplet size and oil droplet distribution were measured by Malvern Mastersizer 2000 (particle RI = 1.494, Dispertant RI = 1.331, Adsorption 0.3 and Analysis Model: general purpose (spherical)). Mean oil droplet size, d(0.5) was found to be 2.02 µm, d(0.9) to be 3.81.

To verify the emulsion stability the oil droplet size was also measured after 24 hours at 70 °C. The oil droplet size d(0.9) was found to be 3.80 µm.

Example 7

A 600 ml beaker with 365 ml boiling demineralised water was placed in a water bath at 80 ° C. 22.8 grams of pepsin modified sugar beet pectin prepared according to Example 2 was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T 50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. The viscosity of the pectin solution was measured at 75 °C (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute). 37.3 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 grams hot

vitamin E acetate oil (DL-α-tocopheryl acetate from BASF AG was added and mixed with Ultra Turrax for 20 minutes at 10,000 rpm. Viscosity of the fresh made emulsion was measured at 75 °C and found to be 255 cps. Oil droplet size and oil droplet size distribution was measured (Malvern Mastersizer 2000, particle RI = 1.494, Dispertant RI = 1.331, Adsorption 0,3 and Analysis Model: general purpose (spherical)). Mean oil droplet size d(0.5) was found to be 1.21 µm and d(0.9) to be 2.35 µm.

To verify the emulsion stability the droplet size was also measured after 24 hours at 70 °C. The oil droplet size d(0.9) was found to be 2.16 µm.

As will appear from these results the mean oil droplet size was smaller and hence the emulsifying properties (including the emulsion stabilizing properties) of the composition of the invention were superior to those of the composition of Comparative Example 2.

Comparative Example 3

A 600 ml beaker with 360 ml boiling water was placed in a water bath at 80 ° C. 22,8 grams of sugar beet pectin (GENU® Beta pectin, lot 30003, produced by CP Kelco, Grossenbrodde, Germany) was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 37.3 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 gram hot vitamin E acetate oil (DL-α-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the freshly made emulsion was measured at 75 ° C and found to be 741 cP. Particle size and distribution was measured by Malvern Mastersizer 2000, Accessory name: hydro 200 G (A), particle RI: 1,494, Adsorption: 0,3; Analysis model: general purpose (spherical). Mean droplet

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size d(0.5) was found to be 1,85 µm and droplet size distribution d(0.9) to be 3,55 µm.

To verify the emulsion stability the droplet size distribution was also measured after 24 hours at 70 ° C and d(0.9) remained constant : 3,65 µm

Example 8

A 600 ml beaker with 360 ml boiling water was placed in a water bath at 80 ° C. 22,8 grams of carboxypeptidase B modified sugar beet pectin as described in Example 3 was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 37.3 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 gram hot vitamin E acetate oil (DL-alfa-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the freshly made emulsion was measured at 75 ° C and found to be 309 cP. Particle size and distribution was measured by Malvern Mastersizer 2000, Accessory name: hydro 200 G (A), particle RI : 1,494, Adsorption : 0,3; Analysis model : general purpose(spherical). Mean droplet size d(0.5) was found to be 1,37 micron and droplet size distribution d(0.9) to be 2,48 micron.

To verify the emulsion stability the droplet size distribution was also measured after 24 hours at 70 ° C and d(0.9) remained constant : 2,48 micron.

As will appear from these results the mean oil droplet size was smaller and hence the emulsifying properties (including the emulsion stabilizing properties) of the composition of the invention were superior to those of the composition of Comparative Example 3.

Example 9

A 600 ml beaker with 360 ml boiling water was placed in a water bath at 80 ° C. 22,8 grams of Flavorzyme modified sugar beet pectin as described in Example 4 was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 37.3 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. 20.0 gram hot vitamin E acetate oil (DL-α-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the freshly made emulsion was measured at 75 ° C and found to be 298 cP. Particle size and distribution was measured by Malvern Mastersizer 2000, Accessory name: hydro 200 G (A), particle RI: 1,494, Adsorption: 0,3, Analysis mode: general purpose (spherical). Mean droplet size d(0.5) was found to be 1,30 µm and droplet size distribution d(0.9) to be 2,64 µm.

To verify the emulsion stability the droplet size distribution was also measured after 24 hours at 70 ° C and d(0.9) remained constant : 2,63 µm.

As will appear from these results the mean oil droplet size was smaller and hence the emulsifying properties (including the emulsion stabilizing properties) of the composition of the invention were superior to those of the composition of Comparative Example 3.

Comparative Example 4

The ratio of pectin:sugar:oil in the following recipe was 3:3:2.

A 600 ml beaker with 350 ml boiling water was placed in a water bath at 80 ° C. 23 grams of sugar beet pectin was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at

3000 rpm. 23 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. The gum content in the water/saccharose solution was 6,2 %. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the sugar/water solution was measured at 75 ° C and found to be 1181 cP. 15.3 gram hot vitamin E acetate oil (DL-alfa-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Particle size and distribution was measured by Malvern Mastersizer 2000, Accessory name: hydro 200 G (A), particle RI: 1,494, Adsorption: 0,3; Analysis model: general purpose (spherical). Mean droplet size d(0.5) was found to be 2,25 micron and droplet size distribution d(0.9) to be 5,54 micron.

Example 10

The recipe was formulated after the same guidelines as in Comparative Example 4; recipe: pectin:sugar:oil was 3:3:2

A 600 ml beaker with 350 ml boiling water was placed in a water bath at 80 ° C. 80 grams of carboxypeptidase A modified sugar beet pectin as described in example 5 was dispersed in the water and mixed with Ultra Turrax (Ultra Turrax T50 with a R50 stirring shaft) for 10 minutes at 3000 rpm. 80 grams saccharose was added and mixed with Ultra Turrax for 10 minutes at 3000 rpm. The gum content in the water/saccharose solution was 19%. Viscosity (Brookfield Viscometer LVT spindle 4 factor 100 or spindle 3 factor 20 or spindle 2 factor 5, 60 rpm for 1 minute) of the water/saccharose solution was measured at 75 ° C and found to be 773 cP. 53 gram hot vitamin E acetate oil (DL-alfa-tocopheryl acetate from BASF AG) was added and mixed with Ultra Turrax for 20 minutes at 10.000 rpm. Particle size and distribution was measured by Malvern Mastersizer 2000, Accessory name: hydro 200 G (A), particle RI: 1,494, Adsorption: 0,3; Analysis model: general purpose (spherical). Mean droplet size d(0.5) was found to be 0.82 micron and droplet size distribution d(0.9) to be 3,68 micron.

As will appear from these results the mean oil droplet size was smaller and hence the emulsifying properties of the composition of the invention were superior to those of the composition of Comparative Example 4.

Emulsion test: Beverage emulsions

Comparative example 5

33.0 grams of Ester Gum 8 BG (from Hercules) was dissolved in 67.0 grams of orange oil 8380 NAT (H.N. Fusgaard A/S), by agitating gently for approx. two hours.

22.5 grams of Genu® beta pectin type BETA (CP Kelco) was dissolved in 910.0 ml of water by means of the high-speed mixer Silverson L4R, adding the pectin slowly to very hot water (70-80 ° C). The solution was mixed for 5 minutes to ensure complete dispersion and hydration.

The orange oil phase was added to the pectin solution while continuing mixing with the high-speed mixer. While still mixing, 50 % solution (w/v) of citric acid solution was added until a pH of approx. 3.25 (corresponding to approx. 2-10 ml of citric acid solution).

0.5 ml of sodium benzoate (20 % sol. w/v) was added and pH was adjusted to 3.25 (at 25 ° C) with citric acid and mixed for 15 minutes at full speed in a homogeniser APV (Rannie A/S) in two steps: 200 bar first, 50 bar last.

The emulsifying properties of the orange oil emulsions was investigated as follows:

The emulsions were stored 4 days at 40 ° C. After storing the mean droplet size was measured to be 0.94 µm (Malvern MASTERSIZER 2000, particle RI = 1.494, Dispertant RI = 1.331, Adsorption 0.3 and Analysis Model: general purpose (spherical)).

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Example 11

33.0 grams of Ester Gum 8 BG (from Hercules) was dissolved in 67.0 grams of orange oil 8380 NAT. (H.N. Fusgaard A/S), by agitating gently for approx. two hours.

22.5 grams of the Collupulin modified sugar beet pectin as described in Example 1 was dissolved in 910.0 ml of water by means of the high-speed mixer Silverson L4R, adding the pectin slowly to very hot water (70-80 °C). The solution was mixed for 5 minutes to ensure complete dispersion and hydration.

The orange oil phase was added to the pectin solution while continuing mixing with the high-speed mixer. While still mixing, 50 % sol. w/v of citric acid solution was added until a pH of approx. 3.25 (corresponding to approx. 2-10 ml of citric acid solution).

0.5 ml of sodium benzoate (20 % sol. w/v) was added and pH was adjusted to 3.25 (at 25 °C) with citric acid and mixed for 15 minutes at full speed in a homogeniser APV (Rannie A/S) in two steps: 200 bar first, 50 bar last.

The emulsifying properties of the orange oil emulsions was investigated as follows:

The emulsions were stored 4 days at 40 °C. After storing the mean droplet size was measured to be 0,63 µm (Malvern MASTERSIZER 2000, particle RI = 1.494, Dispertant RI = 1.331, Adsorption 0.3 and Analysis Model: general purpose (spherical)).

As will appear from the results the oil droplet size was significantly smaller and hence the emulsifying properties of the composition of the inventions were far superior to those of the composition of Comparative Example 5.

Foam Stabilization Test: Sugar beet pectin in whipping cream, 35% milk fat

In order to compare the foam stabilising effect of enzymatic modified sugar beet pectin and conventional sugar beet pectin (ref), trials has been conducted in whipping cream with 35% milk fat. The syneresis of the whipped cream was measured as an expression for the stability.

Comparative example 6

In this Comparative Example conventional sugar beet pectin is used at 0.25% (w/w).

1381.65 g of cream 38% (Danish - high pasteurized cream from Arla Foods amba, containing 2.1% protein, 3.2% carbohydrate and 38% fat) and 69.6g of skimmed milk (Danish pasteurized skimmed milk from Arla Foods amba, containing 3.4% protein, 4.8% carbohydrate and 0.1% fat) was weighed into a tared 2000 ml glass beaker. 45.0 g sugar was weighed into a 250ml glass beaker and blended with 3.75g conventional sugar beet pectin (GENU® pectin type BETA: SF H-25 batch 30003 from CP Kelco)

The dry mix was dispersed into the cream/milk blend while stirring continuously with a propeller stirrer at 400 rpm for 5 min. The glass beaker was placed in a water bath at 95°C and the sample was heated to 87-90°C and kept at this temperature for 10 minutes while stirring with a propeller.

The weight was adjusted with de-ionized water to 1500g and cooled in room temperature to 75°C while stirring frequently with a whisk. The cream/milk blend was homogenized in a two-stage homogenizer (Rannie homogenizer lab 1250) at 75°C and 20/10 bar. 1200g ml of the cream/milk blend was cooled to 10°C in a any scraped surface heat exchanger at 100 rpm (water temperature of cooling bath was 1°C). When the temperature had reached

10°C the cream blend was tapped into a 1500 ml glass beaker and placed at 5°C water bath until next day (18-24 hours).

Results:

Whipping process: The next day, 300g of the cream/milk blend was weighed into the bowl and whipped on a Hobart mixer (type N-50) at speed 3 using the whisk. The cream was whipped for 4 min. until optimal and uniform appearance.

% Overrun: A tared plastic beaker with volume at 185 ml was carefully filled with the whipped cream to above the rim and scraped off with a knife. The beaker with the foam was weighed and the net weight was noted. A similar plastic beaker was tared and filled to the rim with the cream/milk base and the weight was noted for calculation of the % overrun of foam according to the following formula:

(Weight of cream/milk base – weight of foam) x 100: weight of foam.

The % overrun was 83 %.

Syneresis test: 100 g of the whipped cream was placed at a flat sieve (diameter 10 cm) and leveled out. The sieve was placed on a tared 1000 ml plastic beaker and after 2 hours storage at ambient temperature (approx 22°C) the plastic beaker was weighed and the syneresis calculated as a percentage loss of 100g of foam. Results for the syneresis test was 13 %.

According to the organoleptic evaluation the whipped cream had a watery mouth feel and lack of body.

Example 12

In this Example modified sugar beet pectin is used at 0.25% (w/w).

1381,65 g of cream 38% (Danish - high pasteurized cream from Arla Foods amba, containing 2.1% protein, 3.2% carbohydrate and 38% fat) and 69.6g of skimmed milk (Danish pasteurized skimmed milk from Arla Foods amba, containing 3.4% protein, 4.8% carbohydrate and 0.1% fat) was weighed into a tared 2000 ml glass beaker. 45.0 g sugar was weighed into a 250ml glass beaker and blended with 3.75g Collupulin modified sugar beet pectin as described in Example 1.

The dry mix was processed as described in Comparative Example 6

Results:

Whipping process: The next day, 300g of the cream/milk blend was weighed into the bowl and whipped on a Hobart mixer (type N-50) at speed 3 using the whisk. The cream was whipped for 4 min 15 sec. until optimal and uniform appearance.

% Overrun: A tared plastic beaker with volume at 185 ml was carefully filled with the whipped cream to above the rim and scraped off with a knife. The beaker with the foam was weighed and the net weight was noted.A similar plastic beaker was tared and filled to the rim with the cream/milk base and the weight was noted for calculation of the % overrun of foam according to the following formula:

(Weight of cream/milk base – weight of foam) x 100: weight of foam.

The % overrun was 76 %.

Syneresis test: 100 g of the whipped cream was placed at a flat sieve (diameter 10 cm) and leveled out. The sieve was placed on a tared 1000 ml

plastic beaker and after 2 hours storage at ambient temperature (approx 22°C) the plastic beaker was weighed and the syneresis calculated as a percentage loss of 100g of foam. Results for the syneresis test was 4 %.

According to the organoleptic evaluation the whipped cream had a very nice, full-bodied mouth feel which was superior to that of comparative Example 6.

Comparative Example 7

In this Comparative Example conventional sugar beet pectin is used at 0.125% w/w.

1381,65g of cream 38% (Danish - high pasteurized cream from Arla Foods amba, containing 2.1% protein, 3.2% carbohydrate and 38% fat) and 71,48g of skimmed milk (Danish pasteurized skimmed milk from Arla Foods amba, containing 3.4% protein, 4.8% carbohydrate and 0.1% fat) was weighed into a tared 2000 ml glass beaker. 45.0 g sugar was weighed into a 250ml glass beaker and blended with 1.88g conventional sugar beet pectin (GENU® pectin type BETA: SF H-25 batch 30003 from CP Kelco).

The dry mix was dispersed into the cream/milk blend while stirring continuously with a propeller stirrer at 400 rpm for 5 min. The glass beaker was placed in a water bath at 95°C and the sample was heated to 87-90°C and kept at this temperature for 10 minutes while stirring with a propeller.

The weight was adjusted with de-ionized water to 1500g and cooled in room temperature to 75°C while stirring frequently with a whisk. The cream/milk blend was homogenized in a two-stage homogenizer (Rannie homogenizer lab 1250) at 75°C and 20/10 bars. 1200g ml of the cream/milk blend was cooled to 10°C in a scraped surface heat exchanger at 100 rpm (water temperature of cooling bath was 1°C). When the temperature had reached

10°C, the cream blend was tapped into a 1500 ml glass beaker and placed at 5°C water bath until next day (18-24 hours).

Results:

Whipping process: The next day, 300g of the cream/milk blend was weighed into the bowl and whipped on a Hobart mixer (type N-50) at speed 3 using the whisk. The cream was whipped for 4 min 30 sec until optimal and uniform appearance .

% Overrun: A tared plastic beaker with volume at 185 ml was carefully filled with the whipped cream to above the rim and scraped off with a knife. The beaker with the foam was weighed and the net weight was noted. A similar plastic beaker was tared and filled to the rim with the cream/milk base and the weight was noted for calculation of the % overrun of foam according to the following formula:

(Weight of cream/milk base – weight of foam) x 100: weight of foam.

The % overrun was found to be 82 %.

Syneresis test: 100 g of the whipped cream was placed at a flat sieve (diameter 10 cm) and leveled out. The sieve was placed on a tared 1000 ml plastic beaker and after 2 hours storage at ambient temperature (approx 22°C) the plastic beaker was weighed and the syneresis calculated as a percentage loss of 100g of foam. Results for the syneresis test was 10 %.

Example 13

In this Example modified sugar beet pectin is used at 0.125% (w/w).

1381,65g of cream 38% (Danish - high pasteurized cream from Arla Foods amba, containing 2.1% protein, 3.2% carbohydrate and 38% fat) and 71,48g of skimmed milk (Danish pasteurized skimmed milk from Arla Foods amba, containing 3.4% protein, 4.8% carbohydrate and 0.1% fat) was weighed into a tared 2000 ml glass beaker. 45.0g sugar was weighed into a 250ml glass beaker and blended with 1.88g Collupulin modified sugar beet pectin prepared according to Example 1.

The dry mix was processed as described in Comparative example 7.

Results:

Whipping process: The next day, 300g of the cream/milk blend was weighed into the bowl and whipped on a Hobart mixer (type N-50) at speed 3 using the whisk. The cream was whipped for 4 min 15 sec until optimal and uniform appearance.

% Overrun: A tared plastic beaker with volume at 185 ml was carefully filled with the whipped cream to above the rim and scraped off with a knife. The beaker with the foam was weighed and the net weight was noted. A similar plastic beaker was tared and filled to the rim with the cream/milk base and the weight was noted for calculation of the % overrun of foam according to the following formula:

(Weight of cream/milk base – weight of foam) x 100: weight of foam.

The % overrun was found to be 79 %.

Syneresis test: 100 g of the whipped cream was placed at a flat sieve (diameter 10 cm) and leveled out. The sieve was placed on a tared 1000 ml plastic beaker and after 2 hours storage at ambient temperature (approx

22°C) the plastic beaker was weighed and the syneresis calculated as a percentage loss of 100g of foam. Results for the syneresis test was 9 %.

As it will appear from the results the % syneresis was decreased significantly when using protease modified beta pectin instead of conventional beta pectin at a use level at 0.25% w/w. This indicates a more effective stabilization power. At a lower use level - 0.125% w/w, the difference was less significant.

Patent Claims

1. Modified pectic substance, characterized in that it is obtainable by treating a pectic substance from beetroot with a proteolytic enzyme selected from the group consisting of proteases and peptidases.

2. Modified pectic substance according to claim 1, characterized in that it is obtainable by treating the pectic substance from beetroot with papain and pepsin.

3. Modified pectic substance according to claim 2, characterized in that it is obtainable by treating the pectic substance from beetroot with an endopeptidase, such as papain, e.g. Collupulin®.

4. Modified pectic substance according to claim 1, characterized in that it is obtainable by treating the pectic substance from beetroot with an exopeptidase, such as carboxypeptidase B.

5. Modified pectic substance according to claim 1, characterized in that it is obtainable by treating the pectic substance from beetroot with a mixture of endo- and exopeptidases,

6. A process of preparing a modified pectic substance, characterized in treating a liquid medium containing a pectic substance from beetroot with a proteolytic enzyme selected from the group consisting of proteases and peptidases.

7. A process according to claim 6, characterized in using sugar beet pectin as the pectic substance.

8. A product comprising a modified pectic substance according to any of the claims 1-5.

9. Emulsifying agent, characterized in that it comprises a modified pectic substance according to any of the claims 1-5.

10. Microencapsulating agent, characterized in that it comprises a modified pectic substance according to any of the claims 1-5.

11. Foam stabilizer, characterized in that it comprises a modified pectic substance according to any of the claims 1-5.

12. A film-forming agent, characterized in that it comprises a modified pectic substance according to any of the claims 1-5.

13. A process of preparing an emulsified product characterized in the use of a modified pectic substance as emulsifier, wherein the modified pectic substance is obtainable by treating a pectic substance from beetroot with a proteolytic enzyme selected from the group consisting of proteases and peptidases.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 02/00239

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23L 1/0524, C12P 19/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23L, C08B, C12N, C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 0070967 A1 (CHR HANSEN A/S), 30 November 2000 (30.11.00), page 3, line 17 - page 4, line 2; page 5, line 5 - line 27 --	1-13
Y	EP 0580252 A2 (QUEST INTERNATIONAL B V), 26 January 1994 (26.01.94) --	1-13
A	File WPI, Derwent accession no 2000-240001, Fuji Seiyu: "Protein dispersion stabilizer for milk drinks is obtained by decomposing water soluble hemicellulose catalysed by pectinase or protease", JP,A,2000032927,20000202, DW200021 --	1-13

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2002	27 -06- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Inger Löfgren/EK Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 02/00239

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9502044 A1 (NOVO NORDISK A/S), 19 January 1995 (19.01.95), page 12, line 13 - line 23; page 13, line 22 - line 33 --	1-13
A	File WPI/Derwent accession no 1986-216609, Taiyo Sangyo:"Liquefied seaweed prod.prepn. - by enzymic dissolution of seaweed cellulose", JP,A,61149075,19860707, DW198633 -- --------	1-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/05/02

International application No.
PCT/DK 02/00239

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1178738 A	13/02/02
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		NZ 267985 A	29/01/97
		US 5854050 A	29/12/98
		US 5998190 A	07/12/99
		US 6190905 B	20/02/01

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